FIG. 1

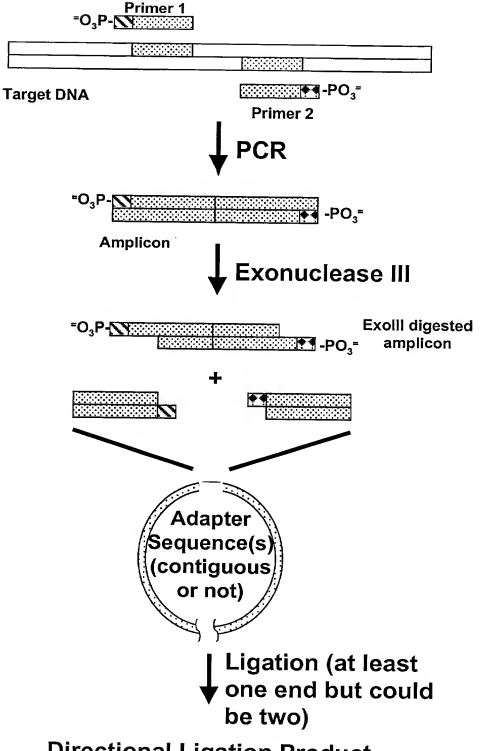
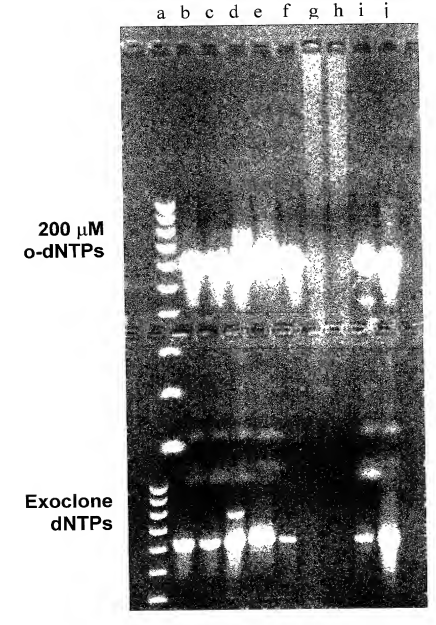


FIG. 2



Lanes:
a) 100 bp ladder
b) Taq
c) REDTaq
d) AccuTaq LA
e) KlenTaq LA
f) Pfu Turbo
g) Vent
h) Deep Vent
i) Pwo
j) UlTma

FIG. 3

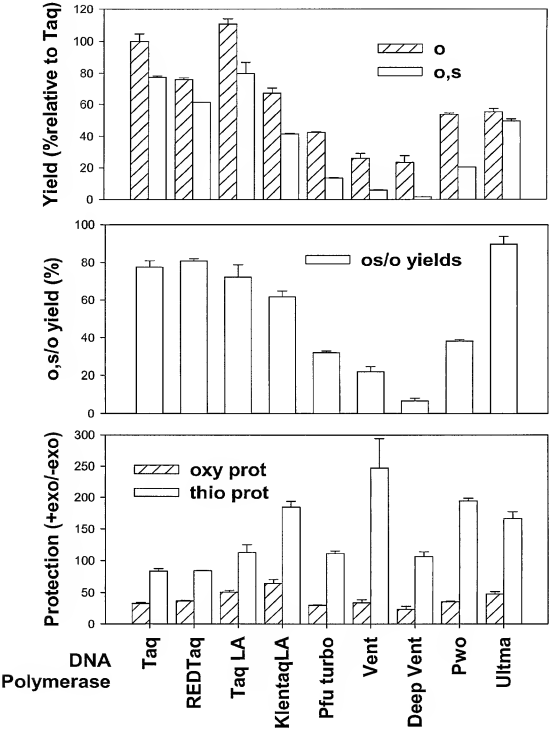

Yield (%relative to Taq)
o
o,s
o,s/o yield (%)
os/o yields
Protection (+exo/-exo)
oxy prot
thio prot
DNA Polymerase
Taq
REDTaq
Taq LA
KlentaqLA
Pfu turbo
Vent
Deep Vent
Pwo
Ultma

FIG. 4

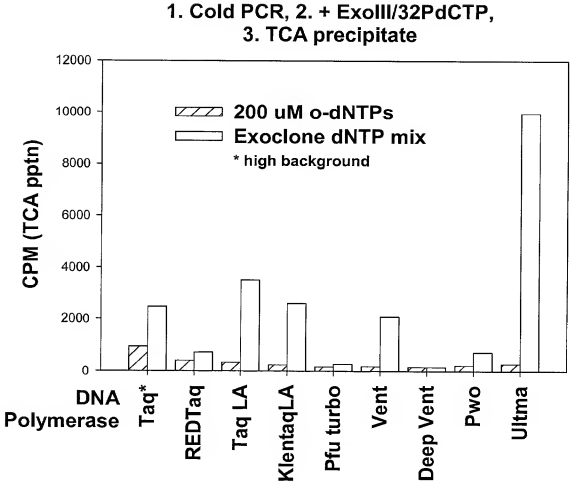


FIG. 5

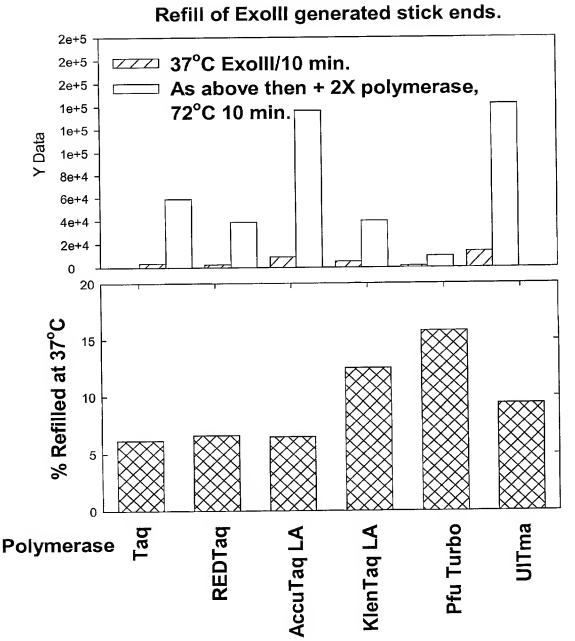

Refill of ExoIII generated stick ends.
37°C ExoIII/10 min.
As above then + 2X polymerase,
72°C 10 min.
Y Data
2e+5
2e+5
2e+5
1e+5
1e+5
1e+5
8e+4
6e+4
4e+4
2e+4
0
% Refilled at 37°C
20
15
10
5
0
Polymerase
Taq
REDTaq
AccuTaq LA
KlenTaq LA
Pfu Turbo
UlTma

FIG. 6

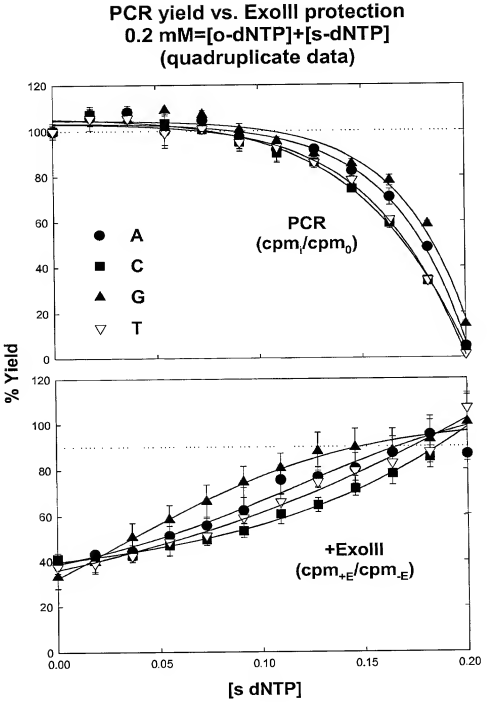

PCR yield vs. ExoIII protection
0.2 mM=[o-dNTP]+[s-dNTP]
(quadruplicate data)
% Yield
PCR
(cpm$_t$/cpm$_0$)
A
C
G
T
+ExoIII
(cpm$_{+E}$/cpm$_{-E}$)
[s dNTP]
0
20
40
60
80
100
120
0.00
0.05
0.10
0.15
0.20

FIG. 7

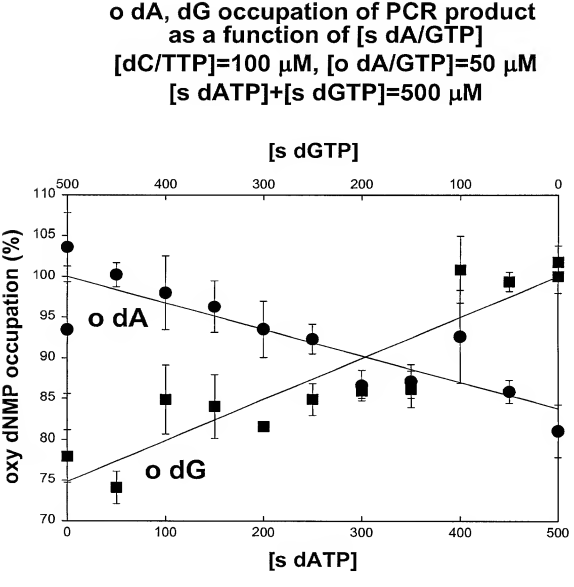

o dA, dG occupation of PCR product
as a function of [s dA/GTP]
[dC/TTP]=100 μM, [o dA/GTP]=50 μM
[s dATP]+[s dGTP]=500 μM
[s dGTP]
500
400
300
200
100
0
oxy dNMP occupation (%)
110
105
100
95
90
85
80
75
70
o dA
o dG
0
100
200
300
400
500
[s dATP]

FIG. 8

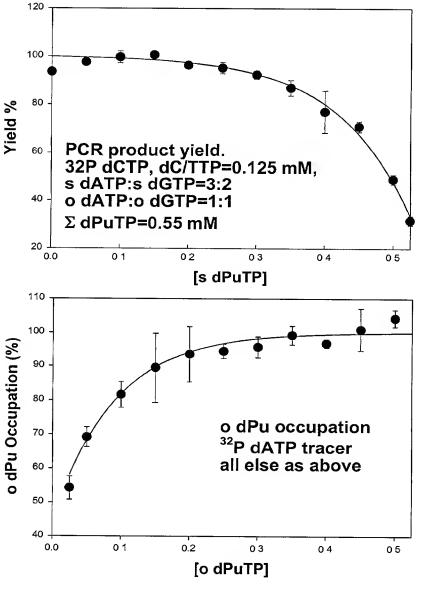

Yield %
PCR product yield.
32P dCTP, dC/TTP=0.125 mM,
s dATP:s dGTP=3:2
o dATP:o dGTP=1:1
Σ dPuTP=0.55 mM
[s dPuTP]
o dPu Occupation (%)
o dPu occupation
32P dATP tracer
all else as above
[o dPuTP]

FIG. 9

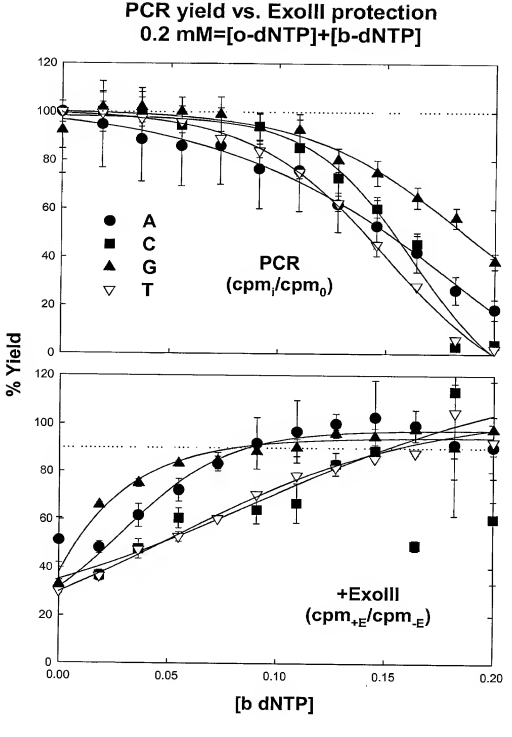
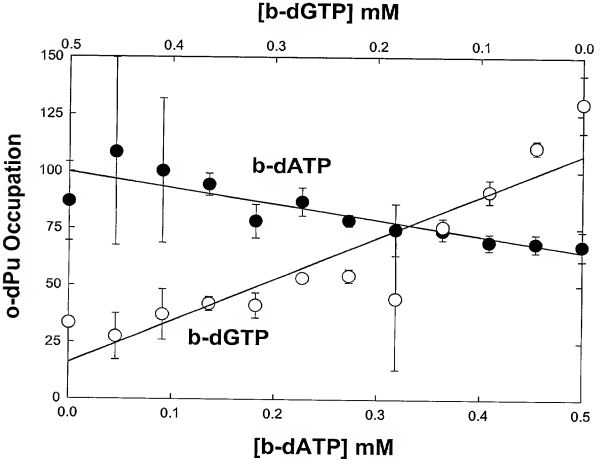

PCR yield vs. ExoIII protection
0.2 mM=[o-dNTP]+[b-dNTP]
% Yield
A
C
G
T
PCR
(cpm$_t$/cpm$_0$)
+ExoIII
(cpm$_{+E}$/cpm$_{-E}$)
[b dNTP]

FIG. 10



o-dA occupation= -70[b-dATP]+99.9
o-dG occupation= -170[b-dGTP]+100
o-dA=o-dG at [b-dATP]/[b-dGTP]=2.5

FIG. 11

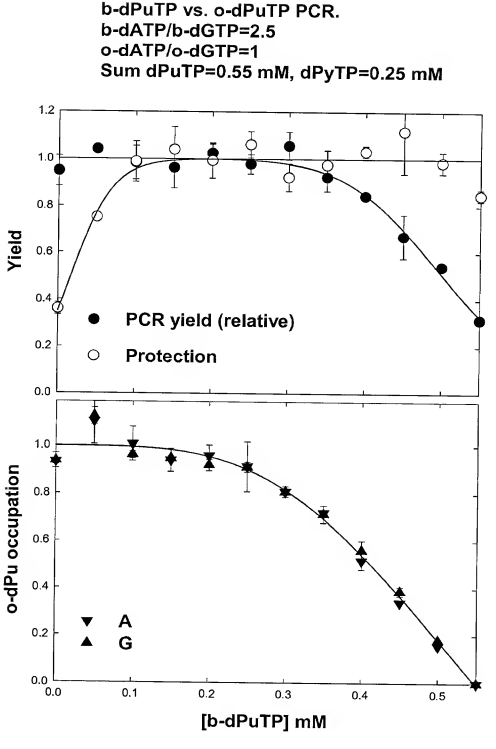
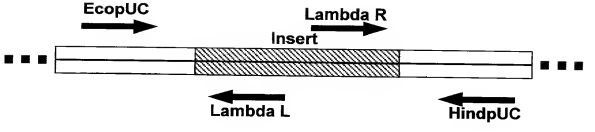
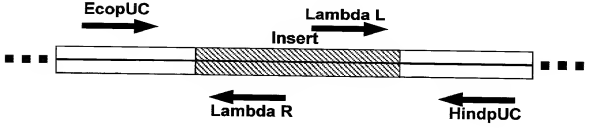

b-dPuTP vs. o-dPuTP PCR.
b-dATP/b-dGTP=2.5
o-dATP/o-dGTP=1
Sum dPuTP=0.55 mM, dPyTP=0.25 mM
Yield
PCR yield (relative)
Protection
o-dPu occupation
A
G
[b-dPuTP] mM

FIG. 12



L-R insertant



R-L insertant

FIG. 13A

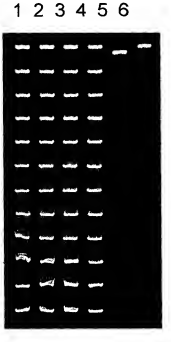


FIG. 13B

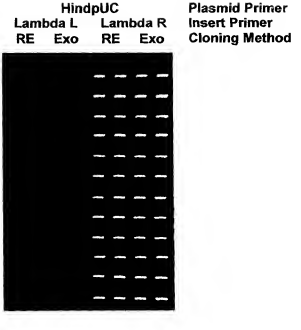


FIG. 14

1. Uncut pBX
2. BamHI pBX
3. XbaI pBX
4. BamHI/XbaI pBX
5. BamHI pUC19
6. XbaI pUC19
7. BamHI/XbaI pUC19
8. Uncut pUC19

FIG. 15

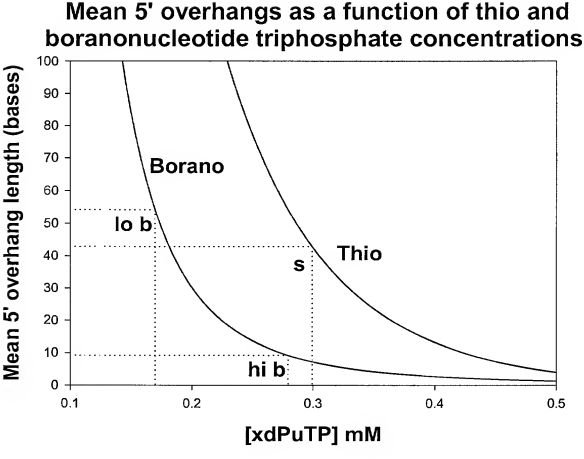

Mean 5' overhangs as a function of thio and boranonucleotide triphosphate concentrations.
Mean 5' overhang length (bases)
100
90
80
70
60
50
40
30
20
10
0
Borano
lo b
Thio
s
hi b
0.1
0.2
0.3
0.4
0.5
[xdPuTP] mM

FIG. 16

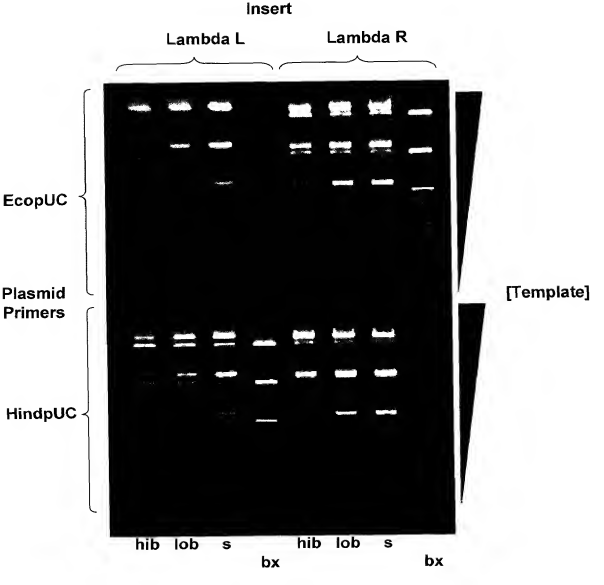

Insert
Lambda L
Lambda R
EcopUC
Plasmid Primers
HindpUC
[Template]
hib lob s
bx
hib lob s
bx
Nucleotide Mix
(bx=control plasmid)

FIG. 17

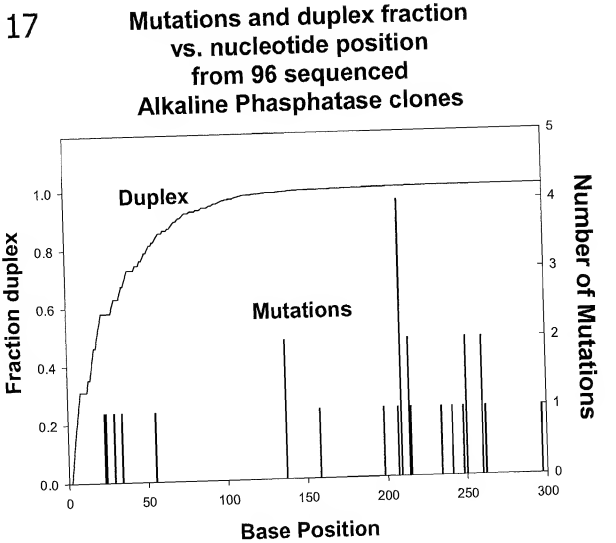


FIG. 18

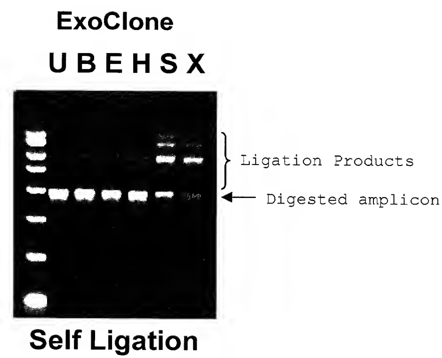

ExoClone
U B E H S X
Ligation Products
Digested amplicon
Self Ligation

FIG.19

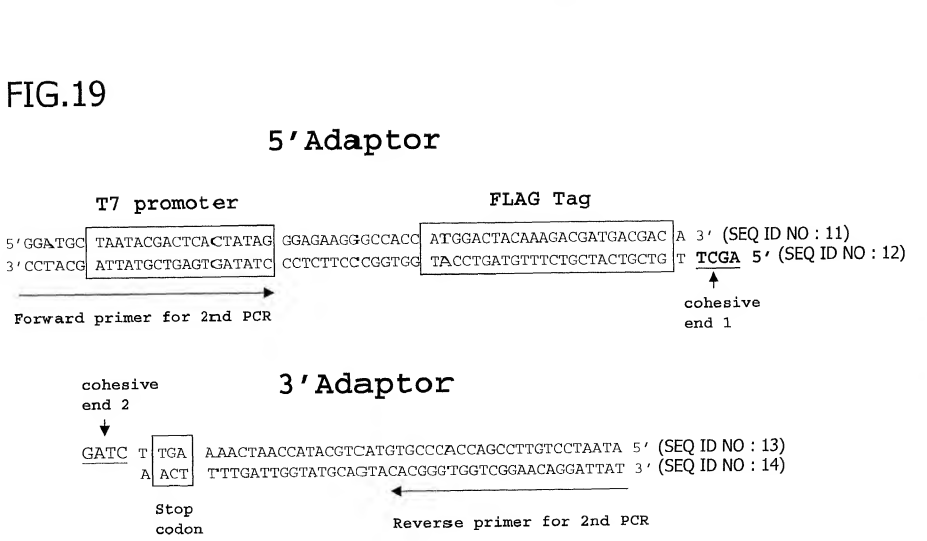

5'Adaptor
T7 promoter
FLAG Tag
5'GGATGC TAATACGACTCACTATAG GGAGAAGGGCCACC ATGGACTACAAAGACGATGACGAC A 3' (SEQ ID NO : 11)
3'CCTACG ATTATGCTGAGTGATATC CCTCTTCCCGGTGG TACCTGATGTTTCTGCTACTGCTG T TCGA 5' (SEQ ID NO : 12)
Forward primer for 2nd PCR
cohesive end 1
3'Adaptor
cohesive end 2
GATC T TGA AAACTAACCATACGTCATGTGCCCACCAGCCTTGTCCTAATA 5' (SEQ ID NO : 13)
A ACT TTTGATTGGTATGCAGTACACGGGTGGTCGGAACAGGATTAT 3' (SEQ ID NO : 14)
Stop codon
Reverse primer for 2nd PCR

FIG. 20